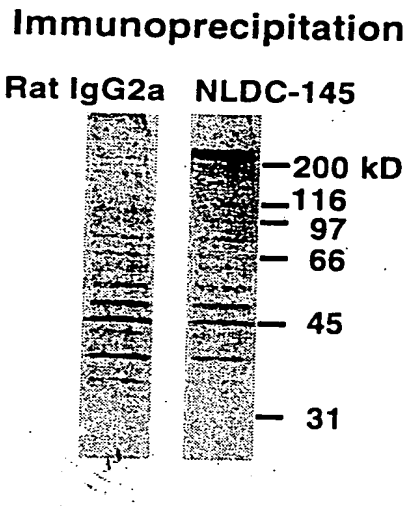
FIGURE 1

A


Immunoprecipitation
Rat IgG2a
NLDC-145
200 kD
116
97
66
45
31

B

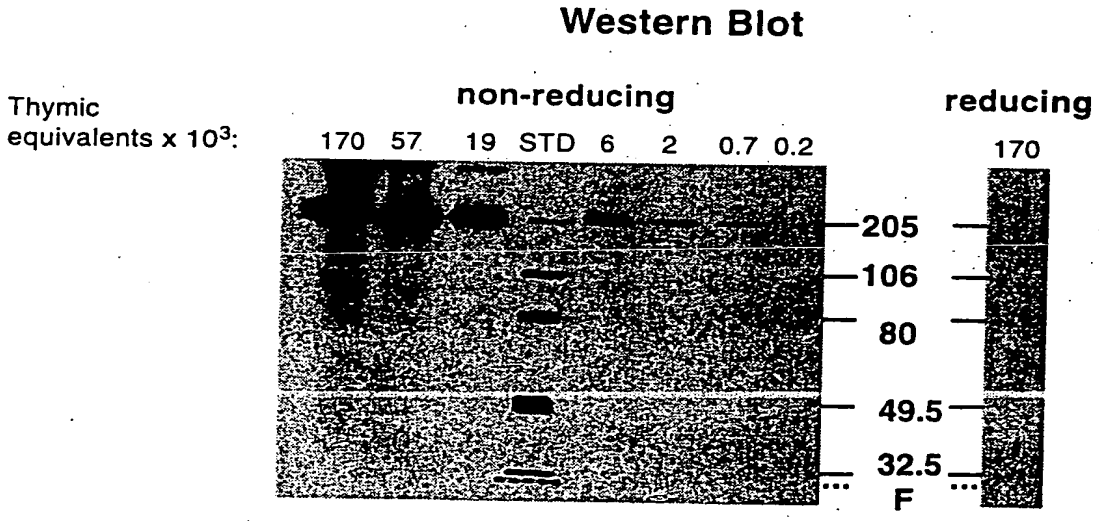

Western Blot
non-reducing
reducing
Thymic equivalents x 10³:
170 57 19 STD 6 2 0.7 0.2
170
205
106
80
49.5
32.5
F

FIGURE 2

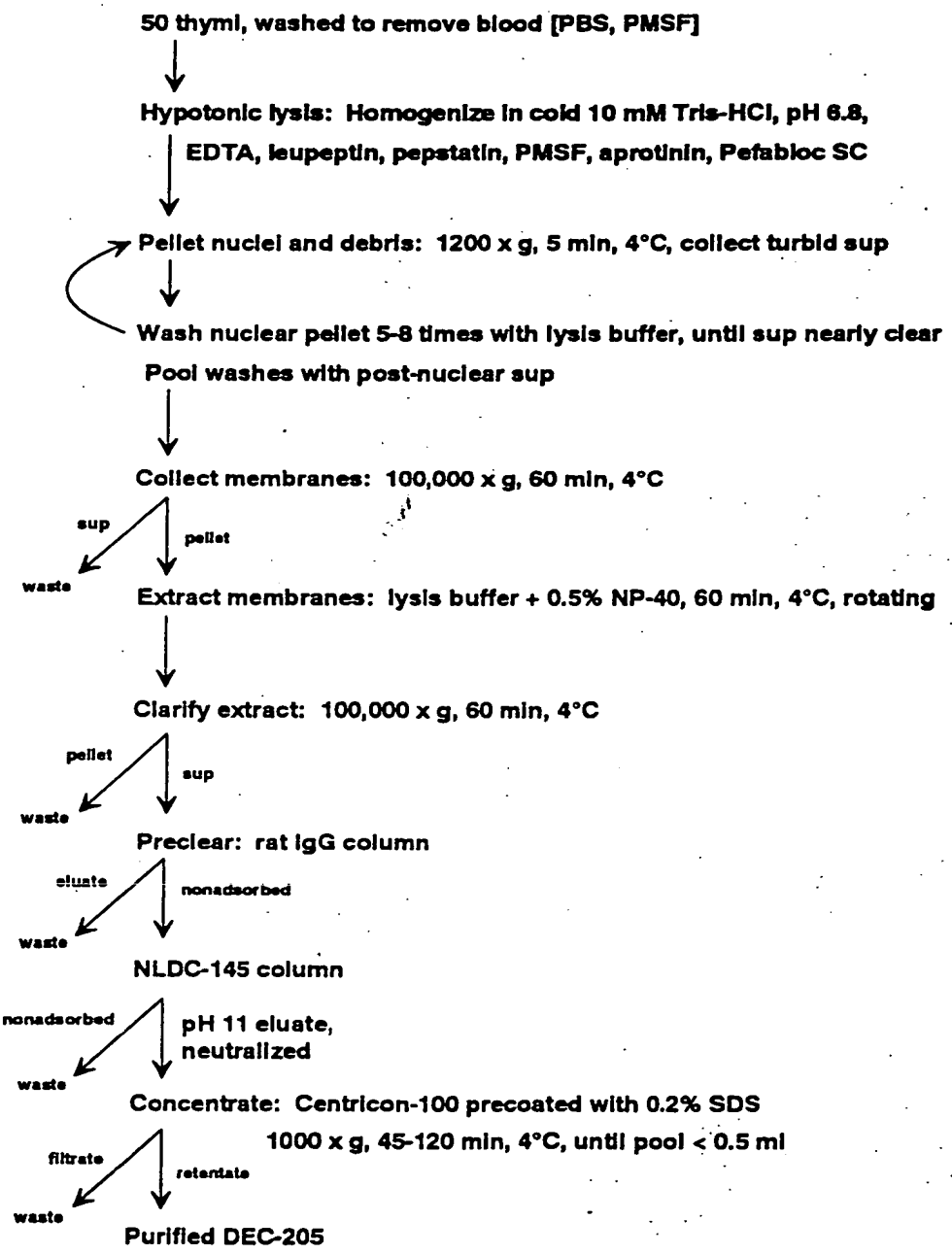

50 thymi, washed to remove blood [PBS, PMSF]
Hypotonic lysis: Homogenize in cold 10 mM Tris-HCl, pH 6.8,
EDTA, leupeptin, pepstatin, PMSF, aprotinin, Pefabloc SC
Pellet nuclei and debris: 1200 x g, 5 min, 4°C, collect turbid sup
Wash nuclear pellet 5-8 times with lysis buffer, until sup nearly clear
Pool washes with post-nuclear sup
Collect membranes: 100,000 x g, 60 min, 4°C
sup
waste
pellet
Extract membranes: lysis buffer + 0.5% NP-40, 60 min, 4°C, rotating
Clarify extract: 100,000 x g, 60 min, 4°C
pellet
waste
sup
Preclear: rat IgG column
eluate
waste
nonadsorbed
NLDC-145 column
nonadsorbed
waste
pH 11 eluate,
neutralized
Concentrate: Centricon-100 precoated with 0.2% SDS
1000 x g, 45-120 min, 4°C, until pool < 0.5 ml
filtrate
waste
retentate
Purified DEC-205

FIGURE 3

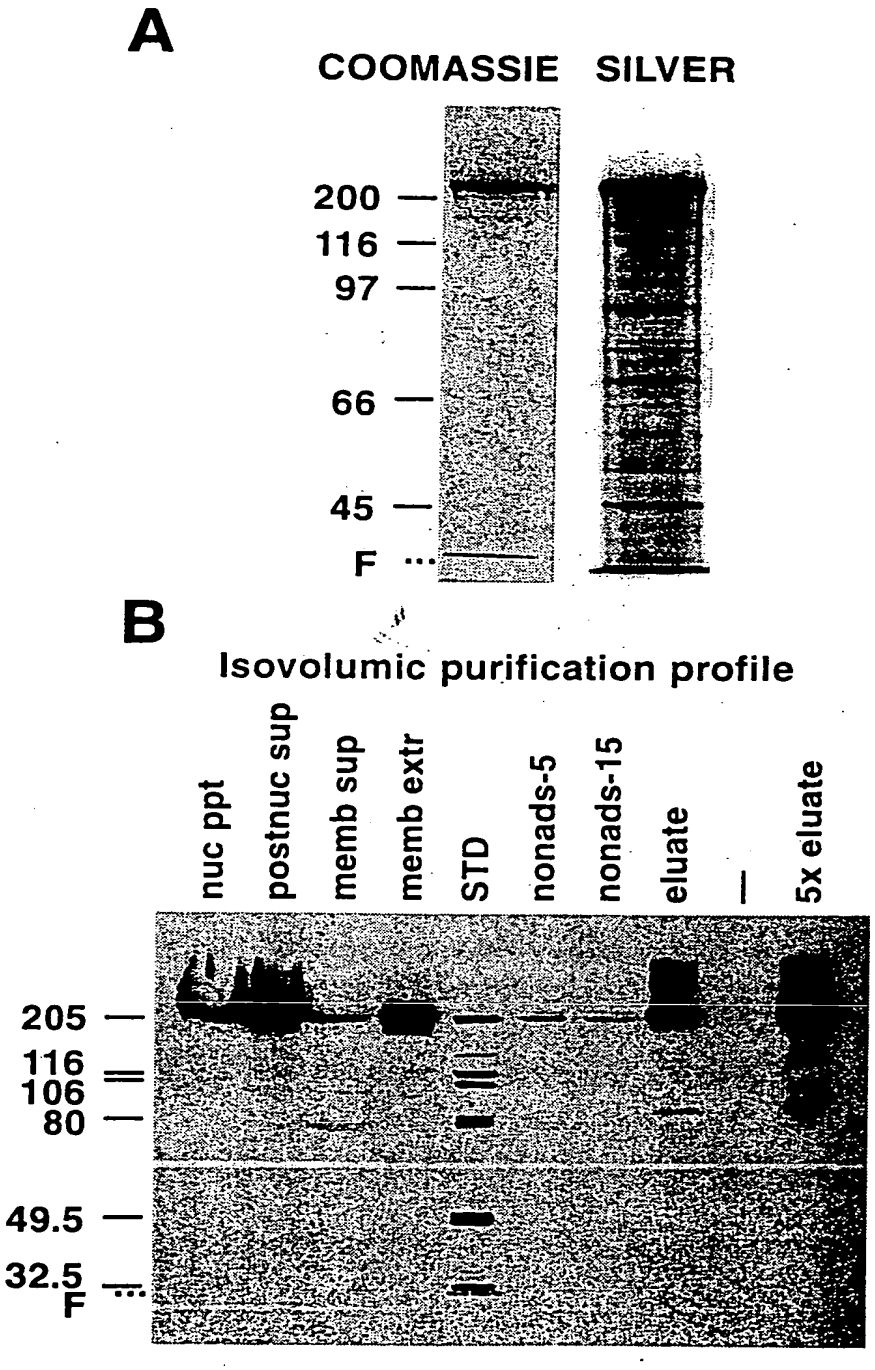

A
COOMASSIE
SILVER
200
116
97
66
45
F
B
Isovolumic purification profile
nuc ppt
postnuc sup
memb sup
memb extr
STD
nonads-5
nonads-15
eluate
—
5x eluate
205
116
106
80
49.5
32.5
F

FIGURE 4

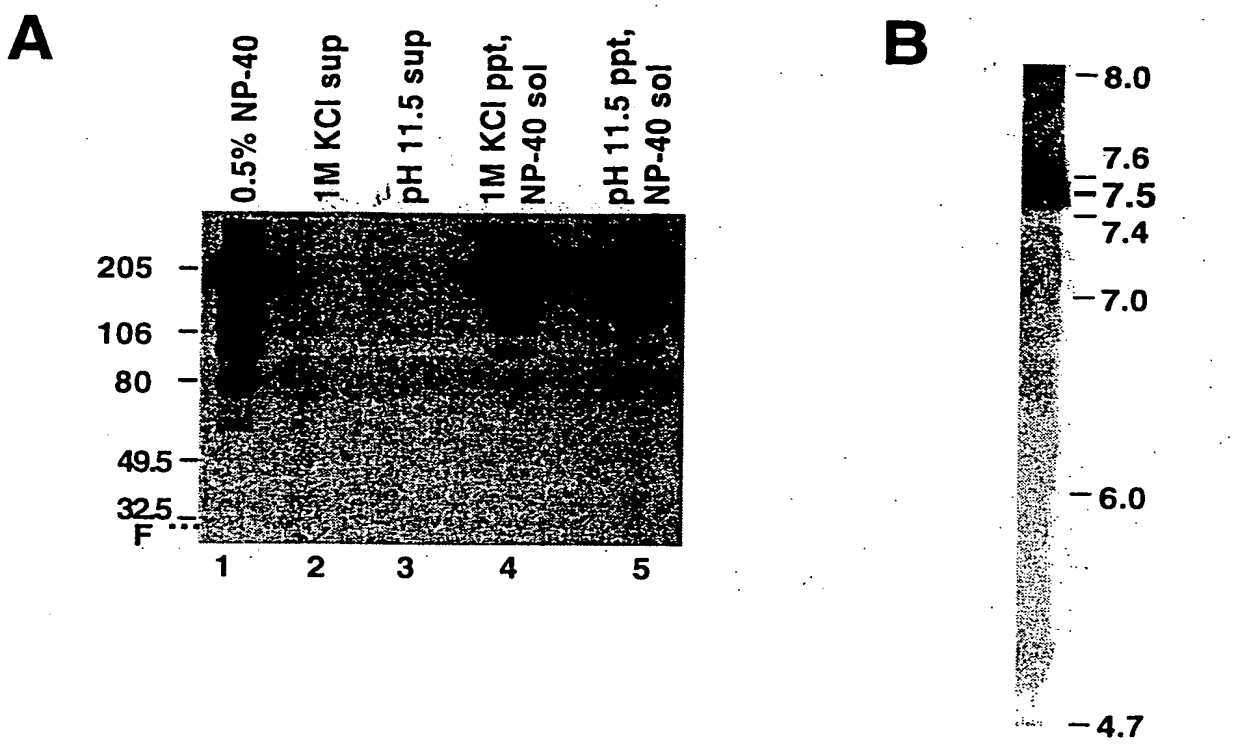
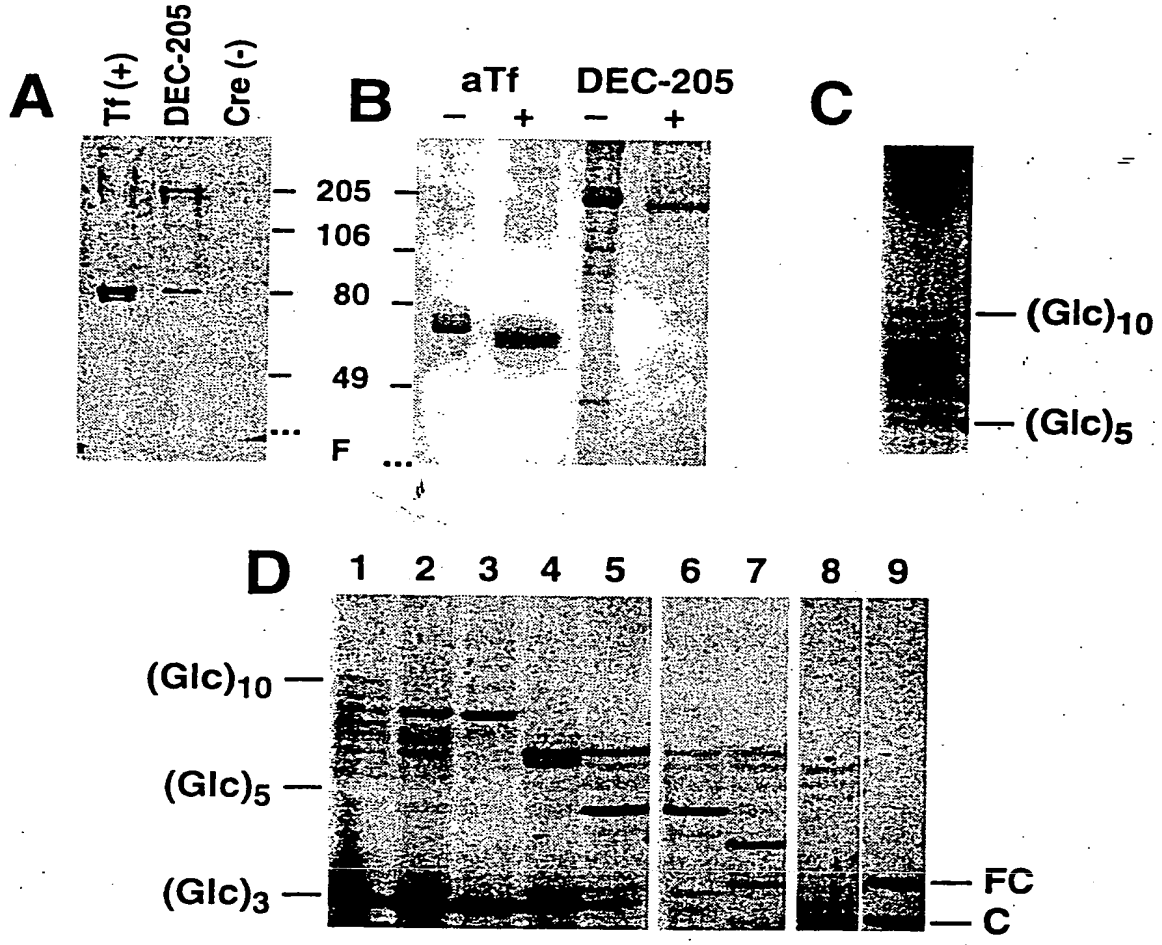

A
0.5% NP-40
1M KCl sup
pH 11.5 sup
1M KCl ppt, NP-40 sol
pH 11.5 ppt, NP-40 sol
205
106
80
49.5
32.5
F
1
2
3
4
5
B
8.0
7.6
7.5
7.4
7.0
6.0
4.7

FIGURE 5


A
Tf (+)
DEC-205
Cre (-)
B
aTf
DEC-205
– + – +
205
106
80
49
F
C
(Glc)10
(Glc)5
D
1 2 3 4 5 6 7 8 9
(Glc)10
(Glc)5
(Glc)3
FC
C

E

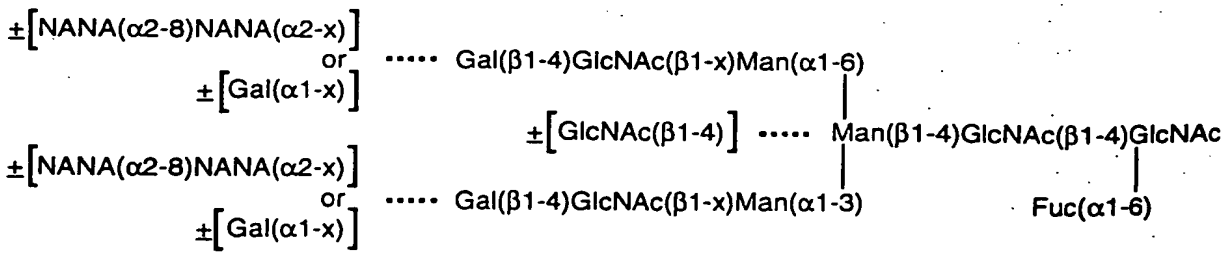

±[NANA(α2-8)NANA(α2-x)]
or
±[Gal(α1-x)]
Gal(β1-4)GlcNAc(β1-x)Man(α1-6)
±[GlcNAc(β1-4)]
Man(β1-4)GlcNAc(β1-4)GlcNAc
±[NANA(α2-8)NANA(α2-x)]
or
±[Gal(α1-x)]
Gal(β1-4)GlcNAc(β1-x)Man(α1-3)
Fuc(α1-6)

FIGURE 6

A

1 5 10 15 20 25

S E S S G N D P F T I V H E N T G K C I Q P L F D

B

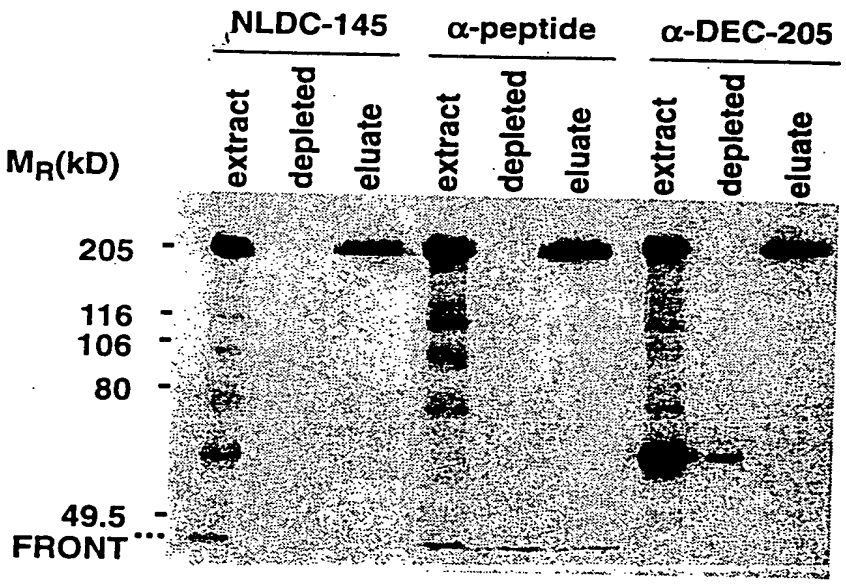
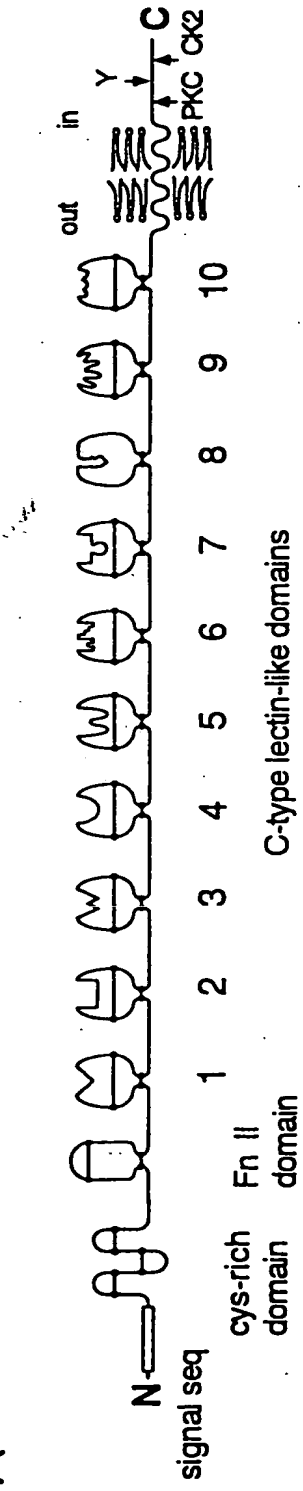


FIGURE 7A


A
N
signal seq
cys-rich domain
Fn II domain
1
2
3
4
5
6
7
8
9
10
C-type lectin-like domains
out
in
Y
PKC
CK2
C

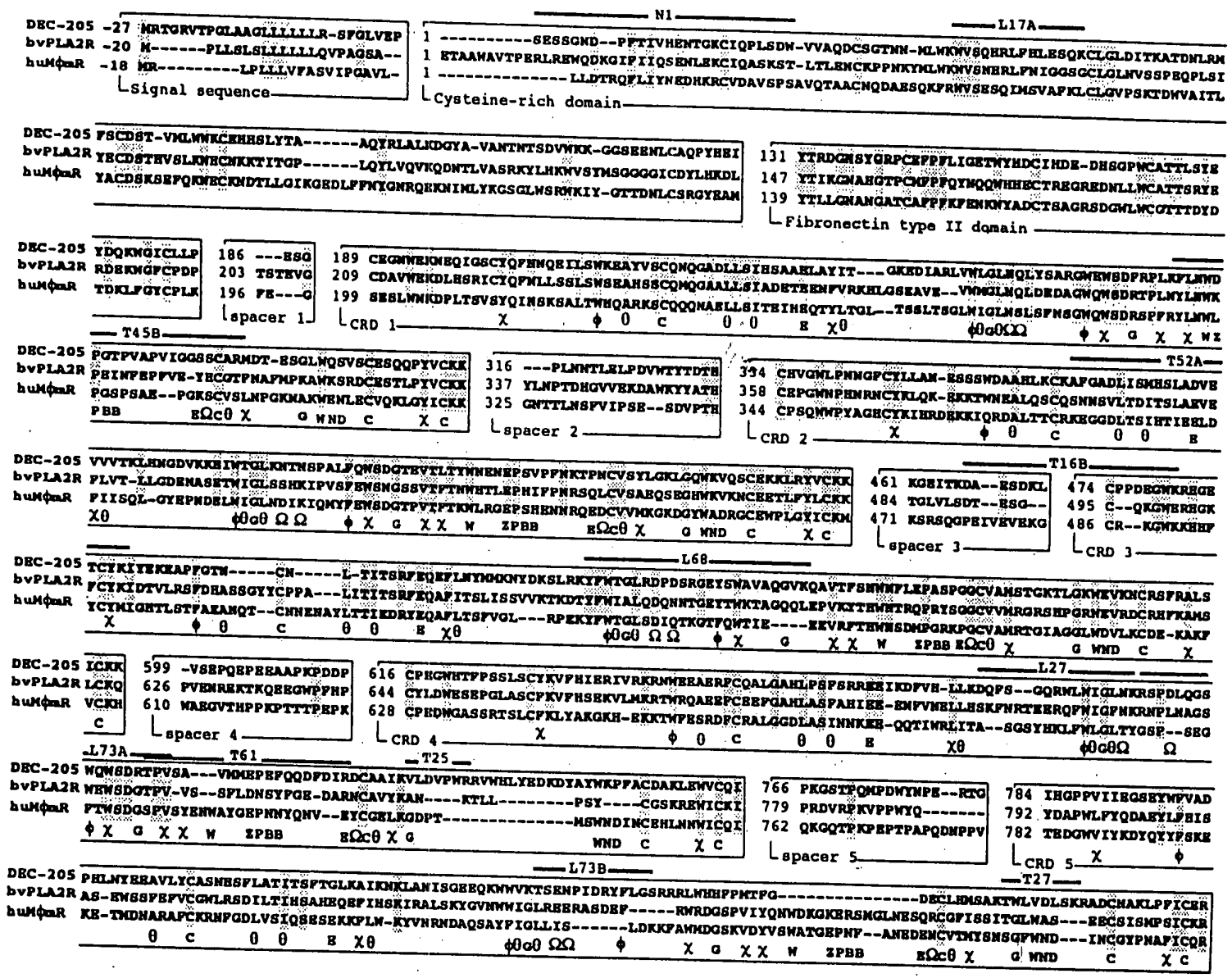


FIGURE 7B

10-1-08 (sheet 8 of 26)

FIGURE 7B cont

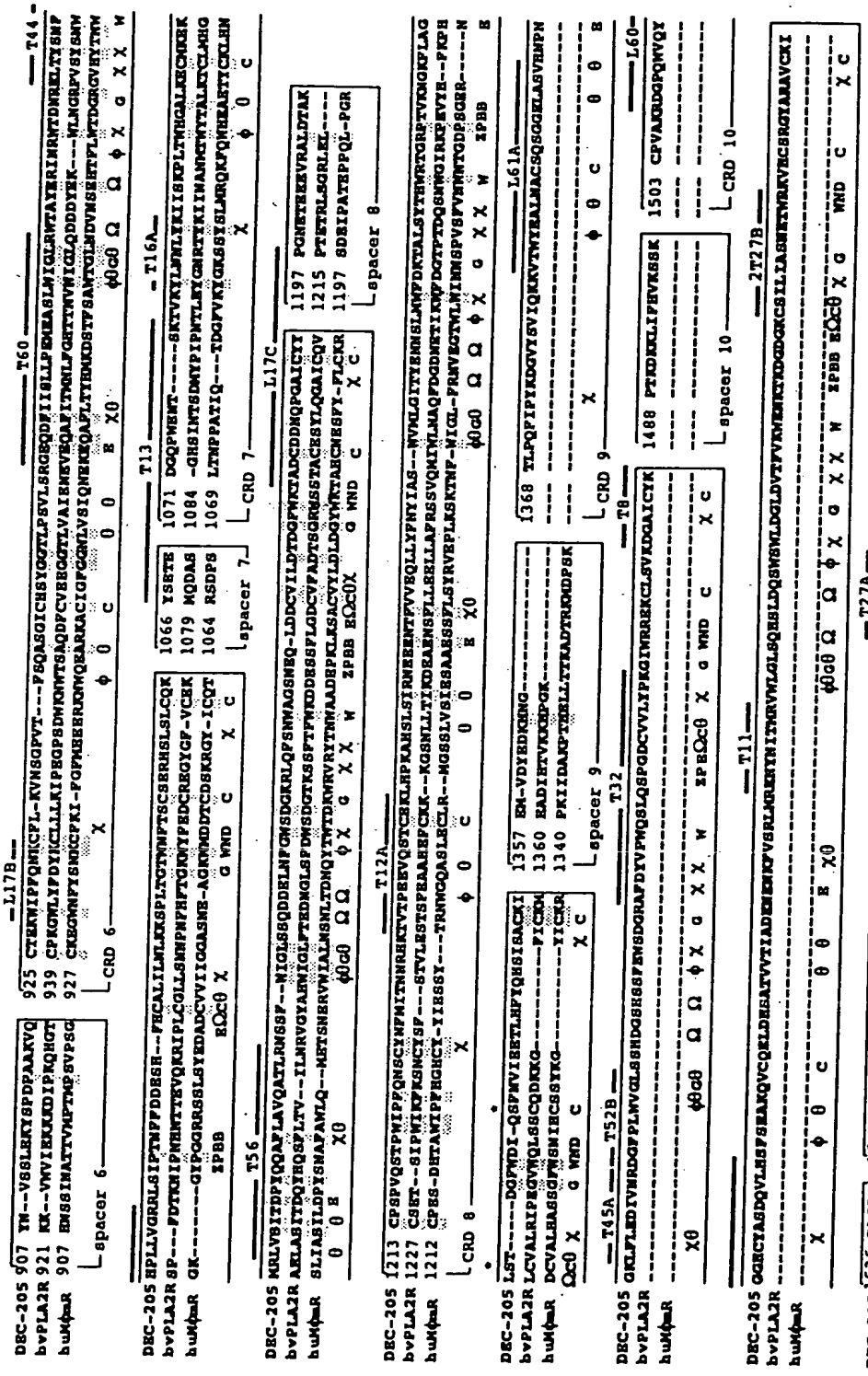


FIGURE 7C

Human

R H *R L H* L *A* G F S S V R Y A *Q* G V N E D E *I* M L P S F H D

R S *H I R W T* G F S S V R Y E *H* G T N E D E *V* M L P S F H D

Mouse

FIGURE 8

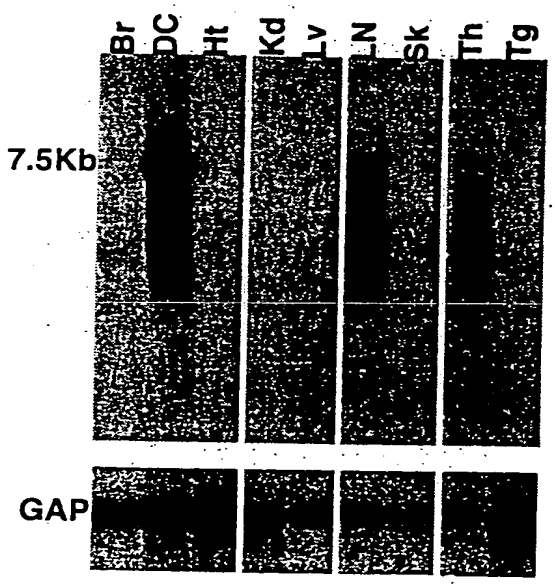


FIGURE 9

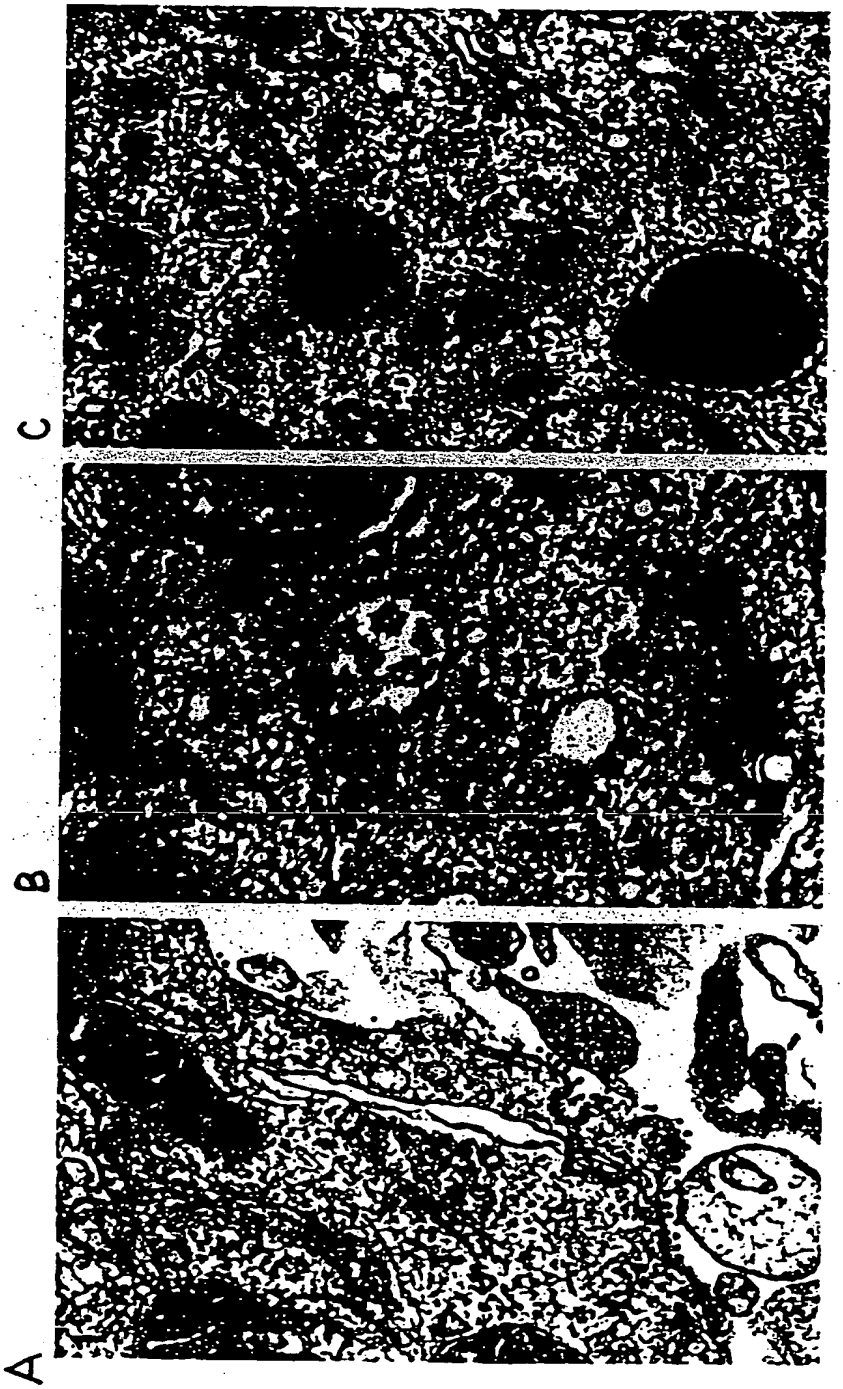


FIGURE 10

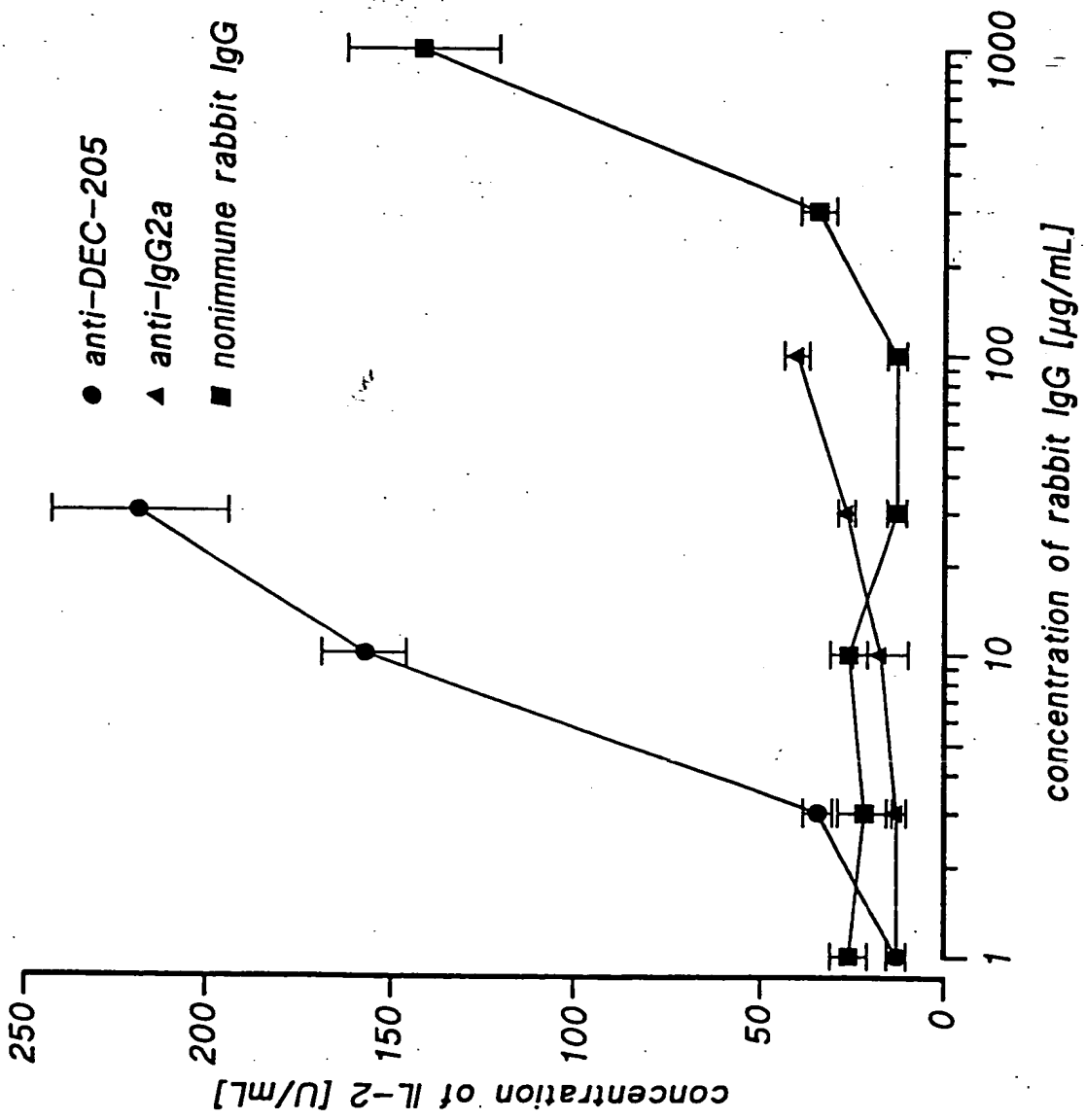

anti-DEC-205
anti-IgG2a
nonimmune rabbit IgG
concentration of IL-2 [U/mL]
concentration of rabbit IgG [µg/mL]
0
50
100
150
200
250
1
10
100
1000

FIGURE 11

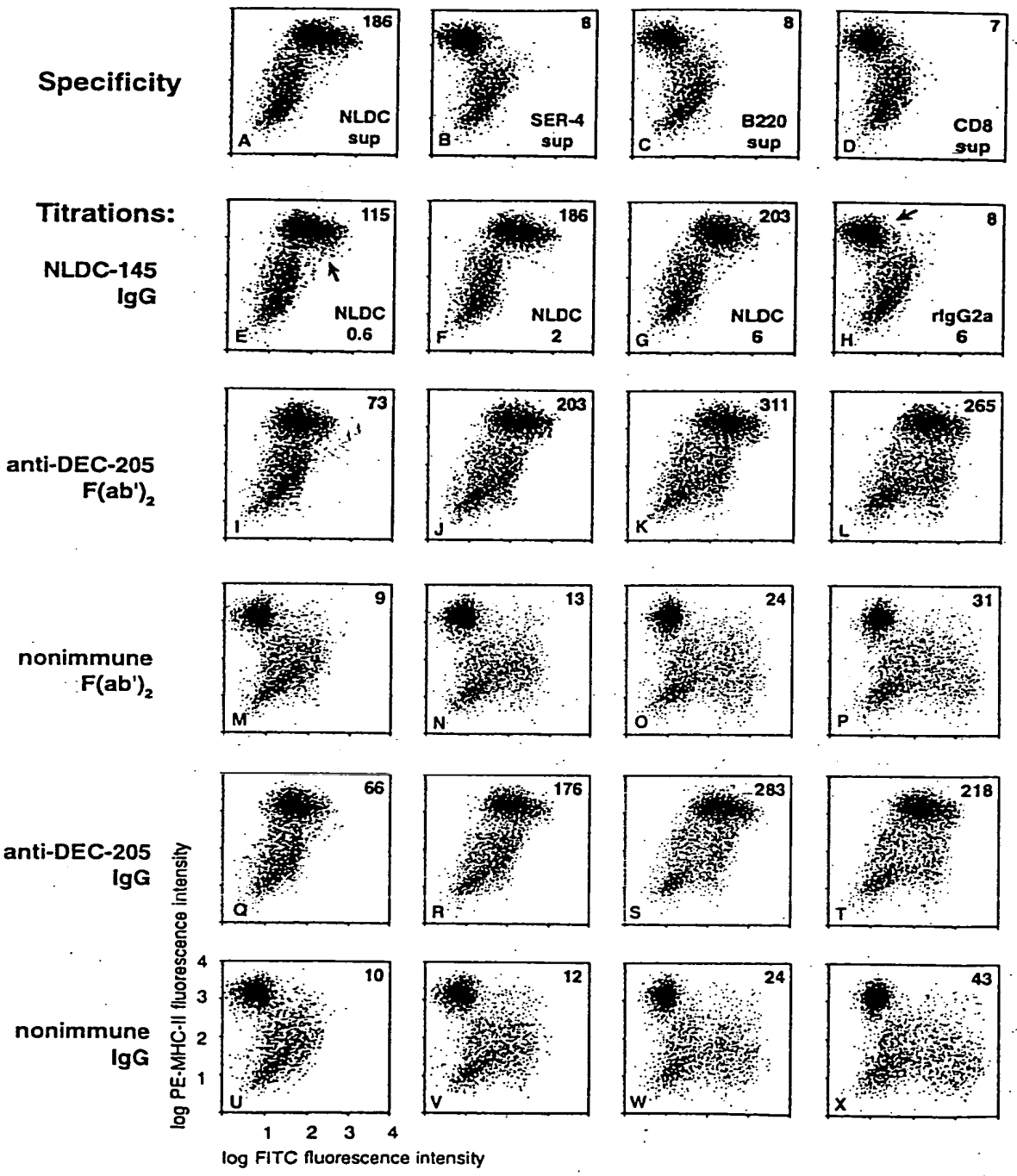

Specificity
Titrations:
NLDC-145 IgG
anti-DEC-205 F(ab')2
nonimmune F(ab')2
anti-DEC-205 IgG
nonimmune IgG
A 186 NLDC sup
B 8 SER-4 sup
C 8 B220 sup
D 7 CD8 sup
E 115 NLDC 0.6
F 186 NLDC 2
G 203 NLDC 6
H 8 rIgG2a 6
I 73
J 203
K 311
L 265
M 9
N 13
O 24
P 31
Q 66
R 176
S 283
T 218
U 10
V 12
W 24
X 43
log PE-MHC-II fluorescence intensity
log FITC fluorescence intensity
1 2 3 4

FIGURE 12

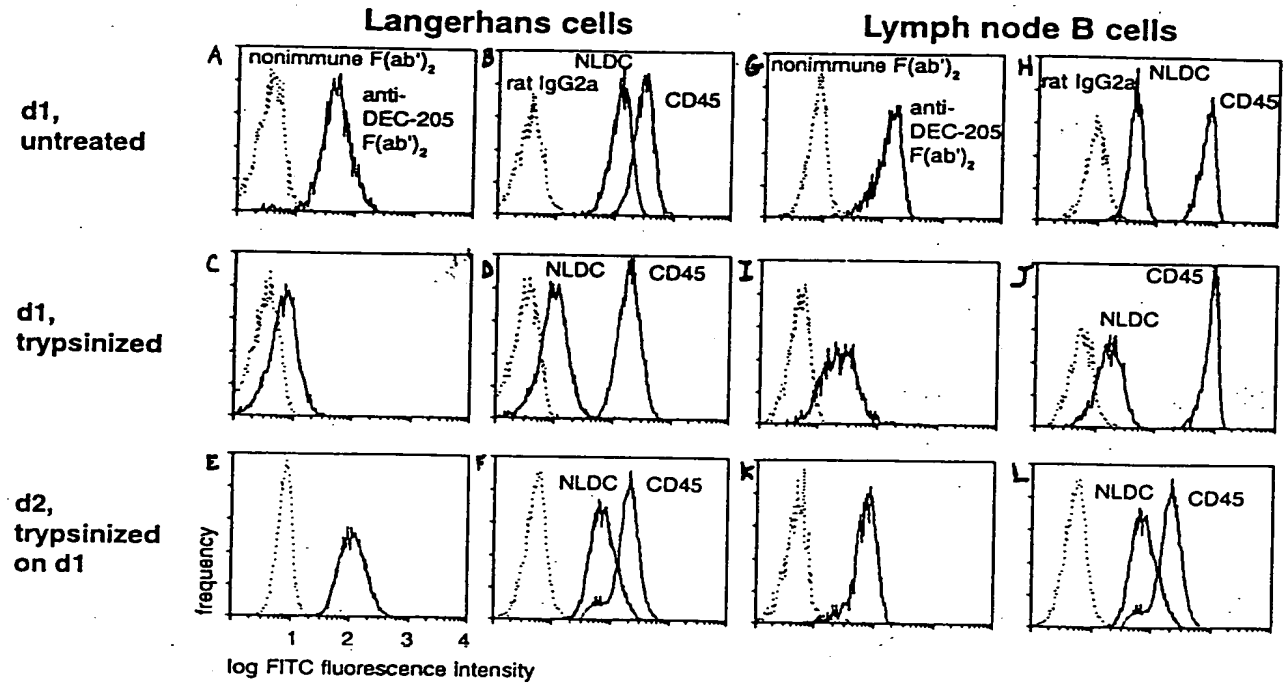

Langerhans cells
Lymph node B cells
d1, untreated
d1, trypsinized
d2, trypsinized on d1
A
nonimmune F(ab')2
anti-DEC-205 F(ab')2
B
NLDC
rat IgG2a
CD45
G
nonimmune F(ab')2
anti-DEC-205 F(ab')2
H
rat IgG2a
NLDC
CD45
C
D
NLDC
CD45
I
J
CD45
NLDC
E
F
NLDC
CD45
K
L
NLDC
CD45
frequency
1
2
3
4
log FITC fluorescence intensity

FIGURE 13

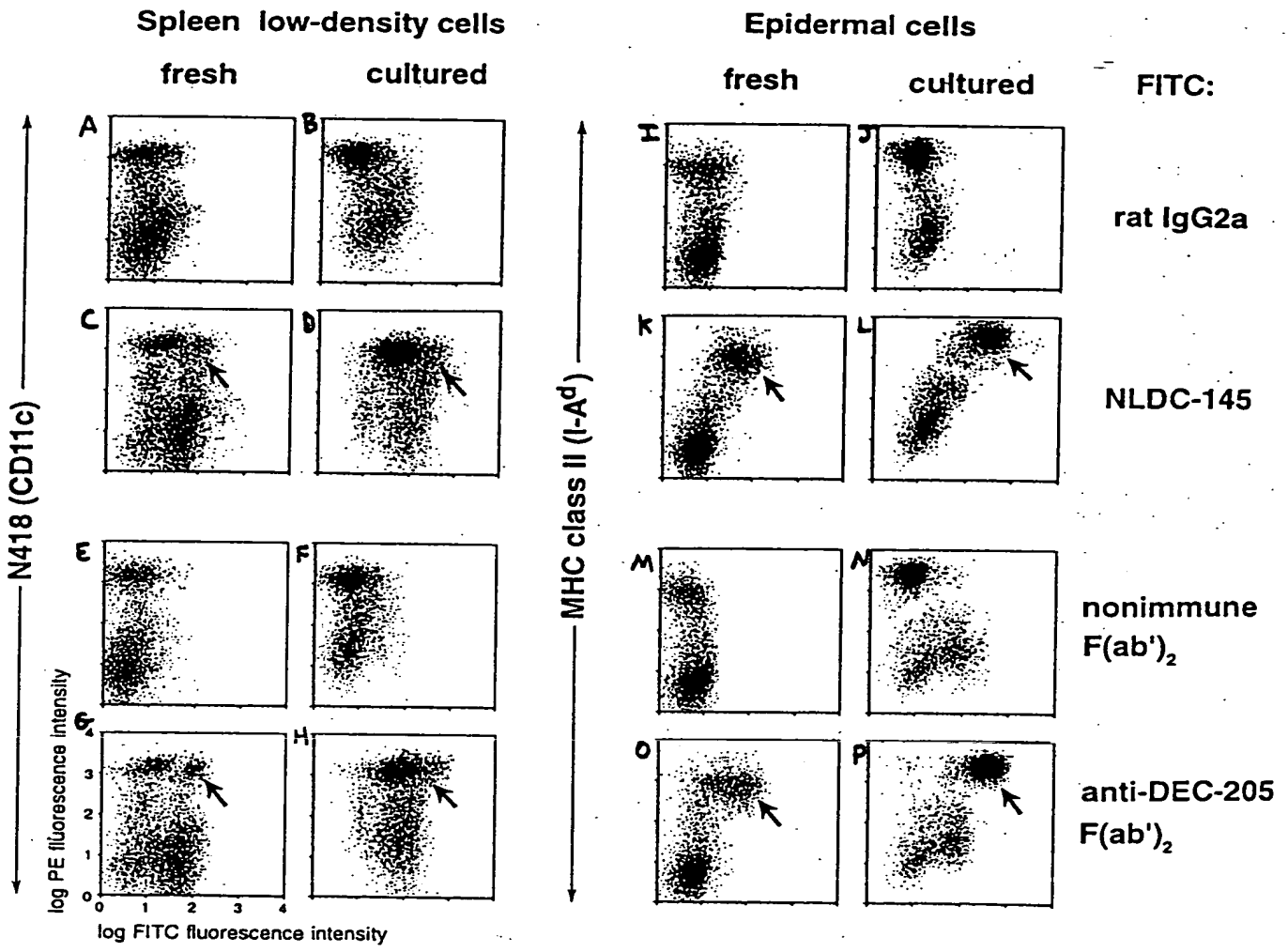

Spleen low-density cells
fresh
cultured
Epidermal cells
fresh
cultured
FITC:
rat IgG2a
NLDC-145
nonimmune F(ab')2
anti-DEC-205 F(ab')2
N418 (CD11c)
MHC class II (I-Ad)
log PE fluorescence intensity
log FITC fluorescence intensity
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P

FIGURE 14

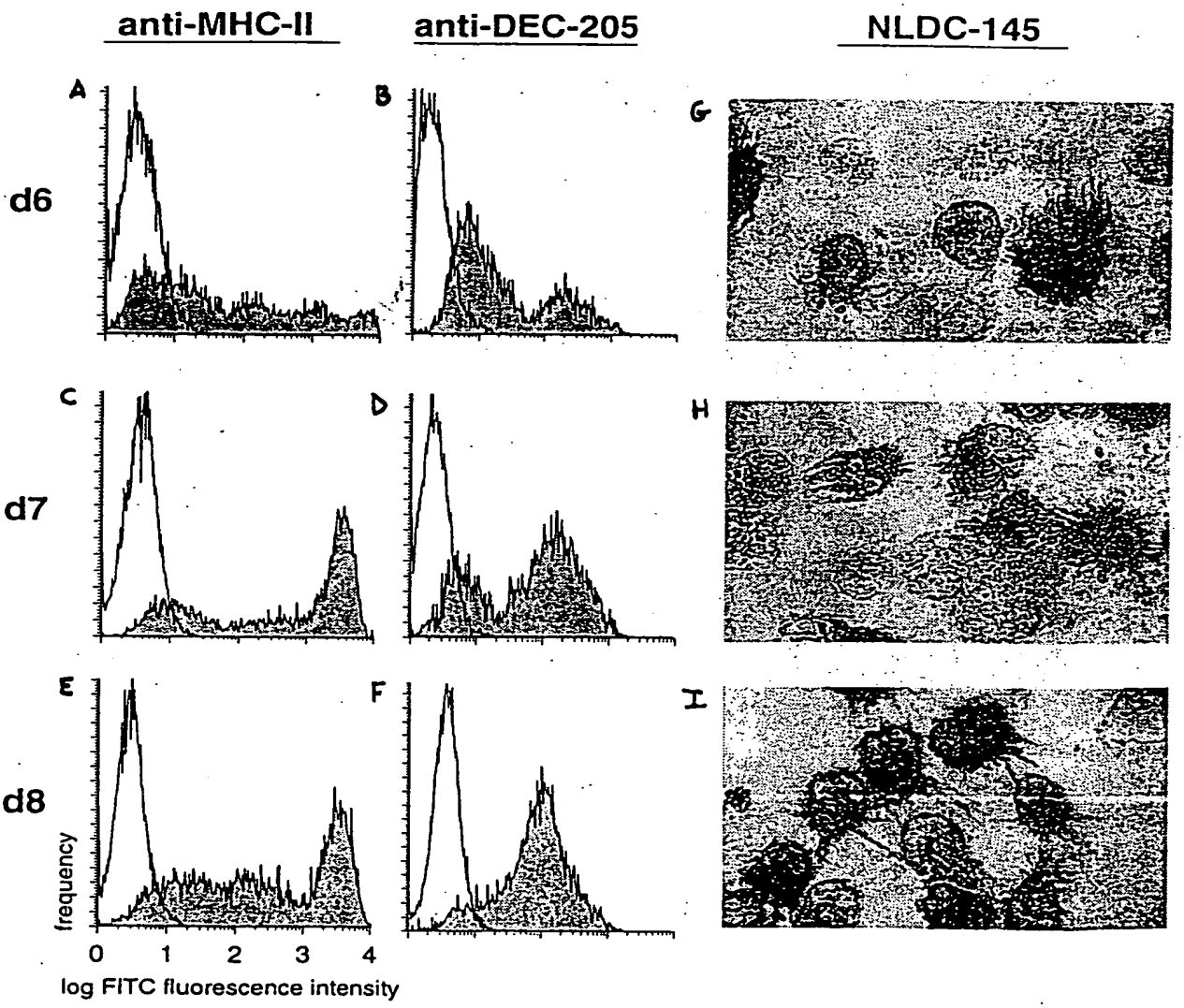

anti-MHC-II
anti-DEC-205
NLDC-145
A
B
G
d6
C
D
H
d7
E
F
I
d8
frequency
0
1
2
3
4
log FITC fluorescence intensity

FIGURE 15

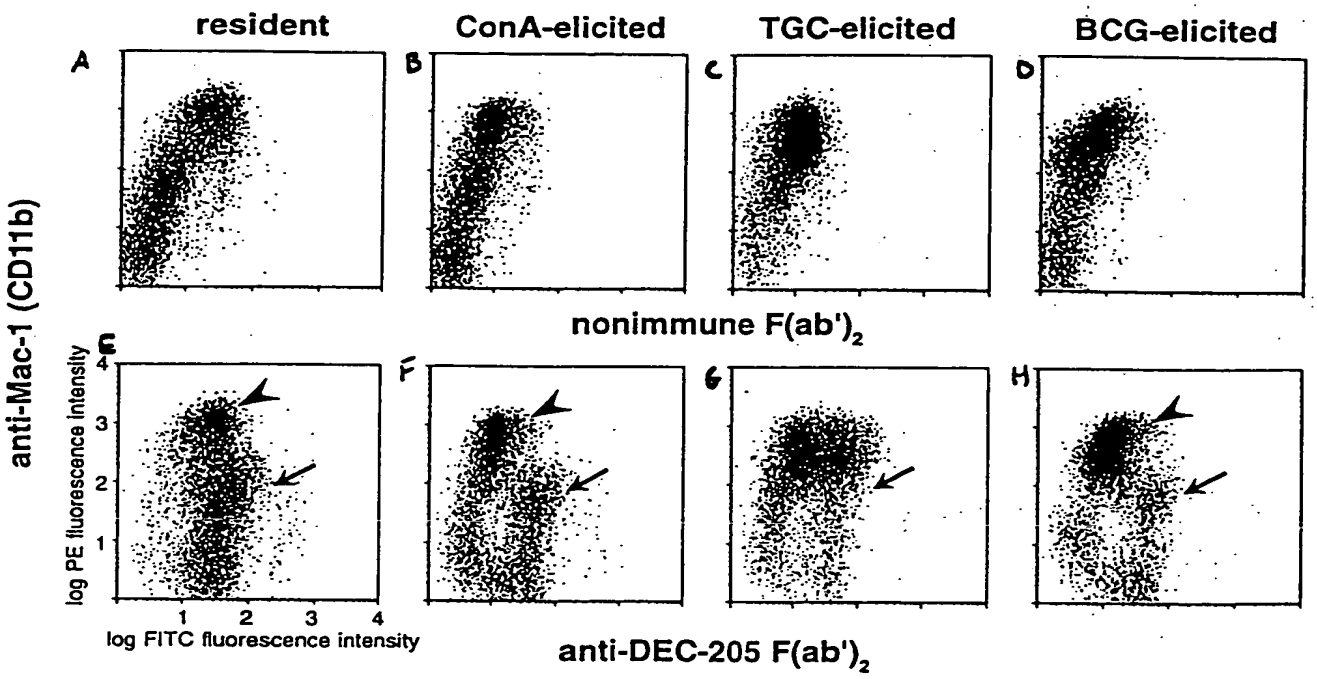

resident
ConA-elicited
TGC-elicited
BCG-elicited
A
B
C
D
E
F
G
H
anti-Mac-1 (CD11b)
nonimmune F(ab')2
anti-DEC-205 F(ab')2
log PE fluorescence intensity
log FITC fluorescence intensity
1
2
3
4

FIGURE 16

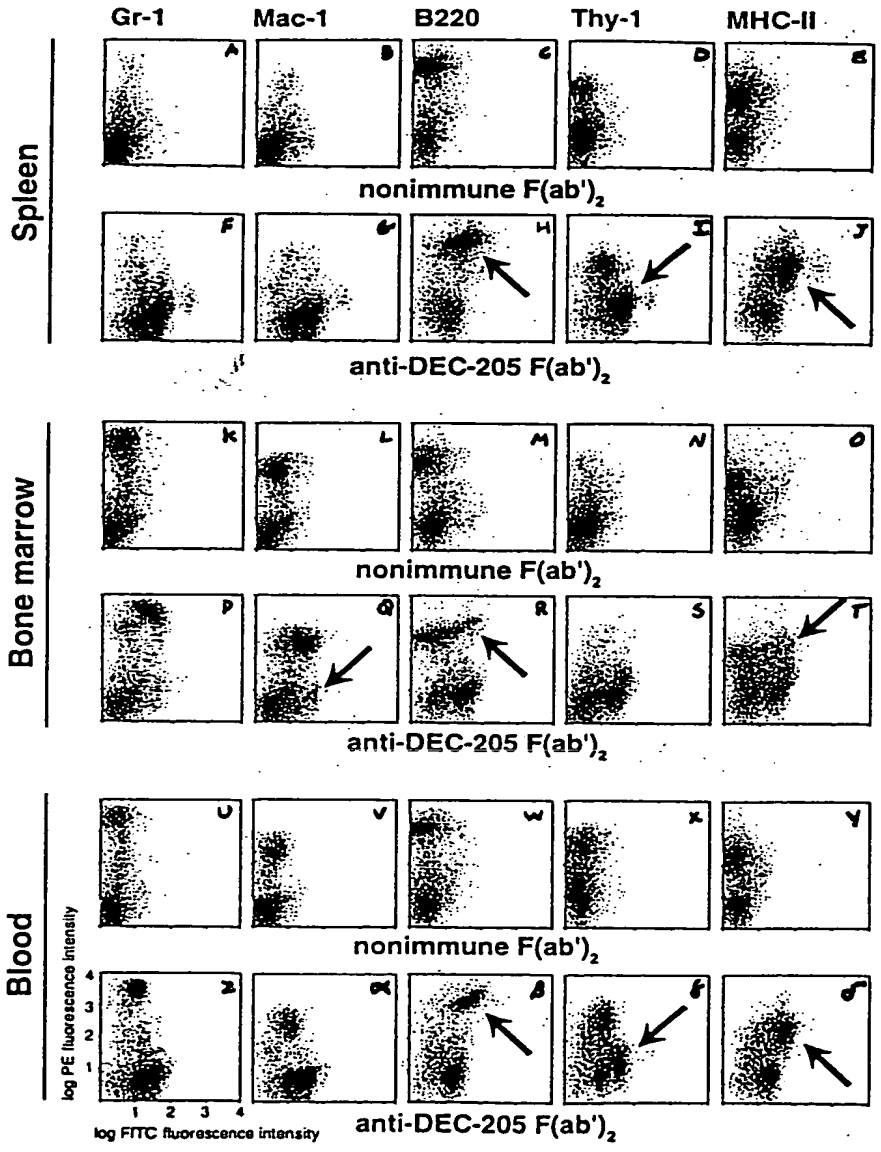

Gr-1
Mac-1
B220
Thy-1
MHC-II
Spleen
nonimmune F(ab')2
anti-DEC-205 F(ab')2
Bone marrow
nonimmune F(ab')2
anti-DEC-205 F(ab')2
Blood
nonimmune F(ab')2
anti-DEC-205 F(ab')2
log PE fluorescence intensity
log FITC fluorescence intensity
1
2
3
4

FIGURE 17

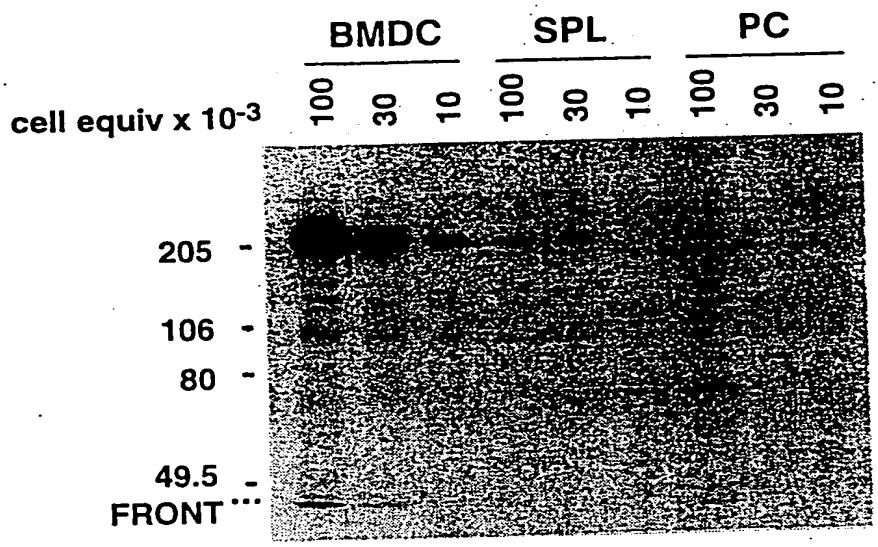

BMDC
SPL
PC
cell equiv x 10-3
100
30
10
100
30
10
100
30
10
205
106
80
49.5
FRONT

FIGURE 18

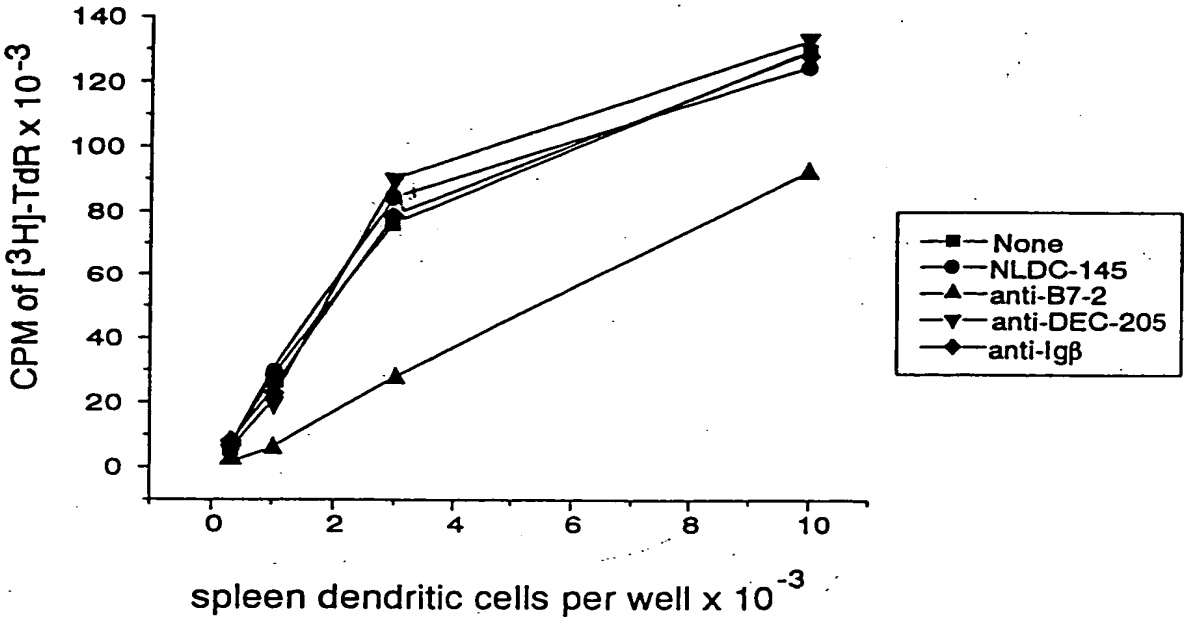

CPM of [3H]-TdR x 10-3
140
120
100
80
60
40
20
0
0
2
4
6
8
10
spleen dendritic cells per well x 10-3
None
NLDC-145
anti-B7-2
anti-DEC-205
anti-Igβ

FIGURE 19

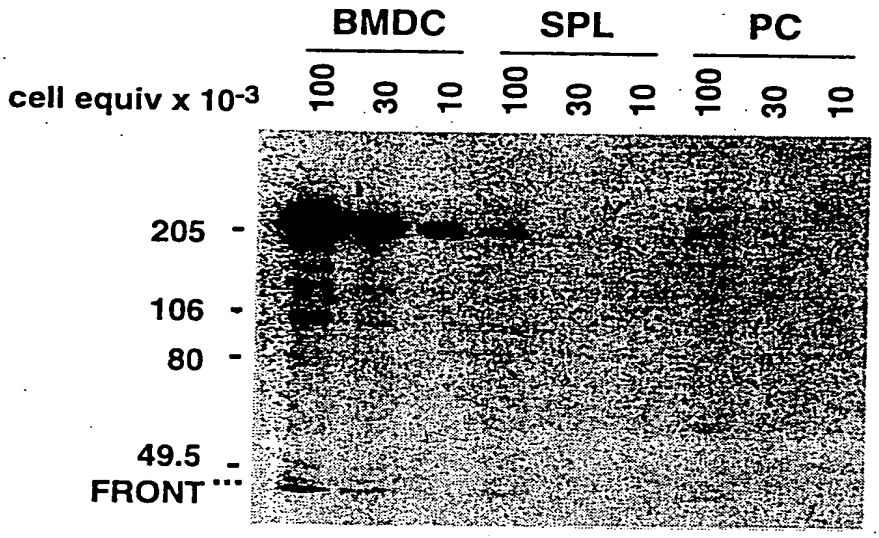

BMDC
SPL
PC
cell equiv x 10-3
100
30
10
100
30
10
100
30
10
205
106
80
49.5
FRONT

FIGURE 20

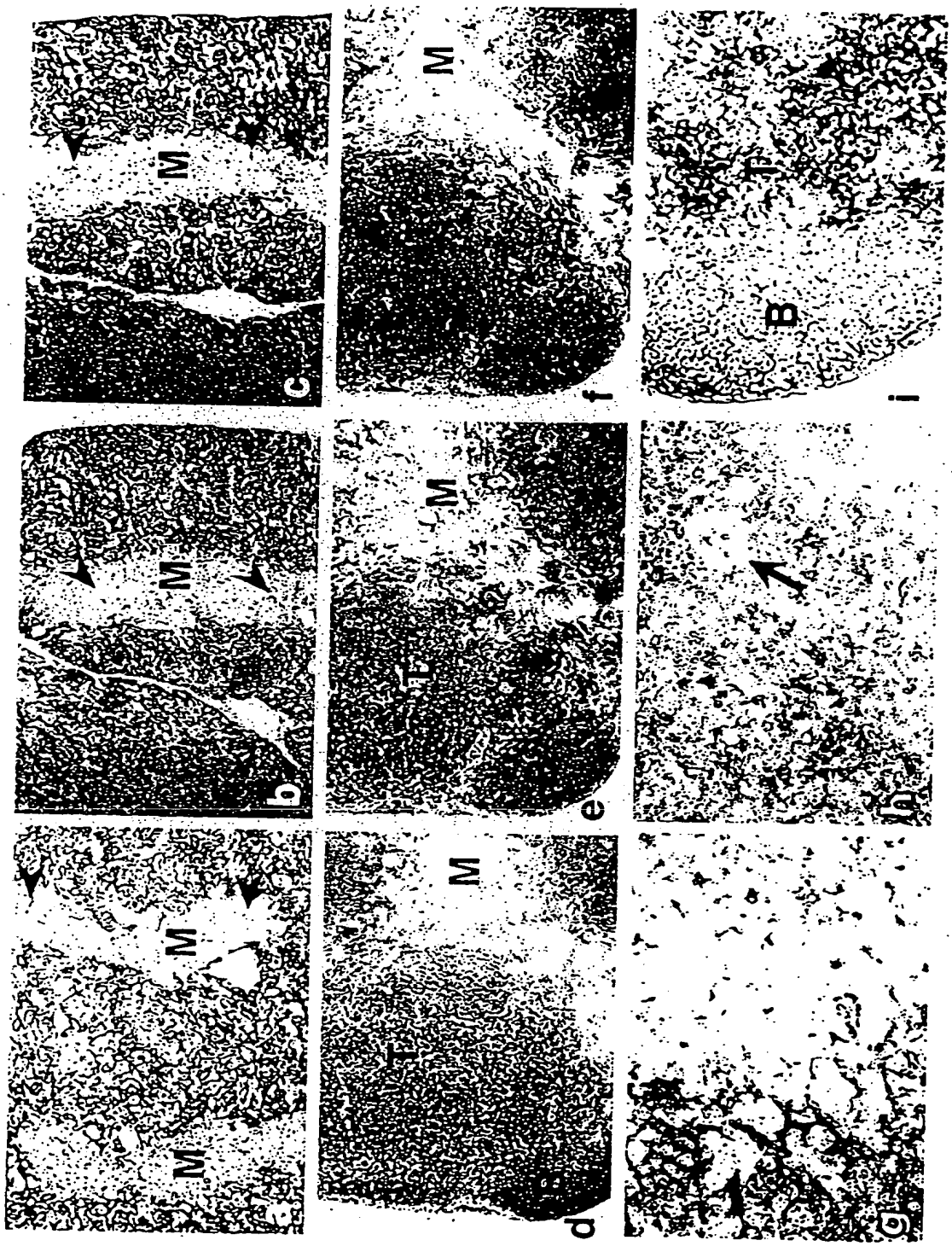


FIGURE 21

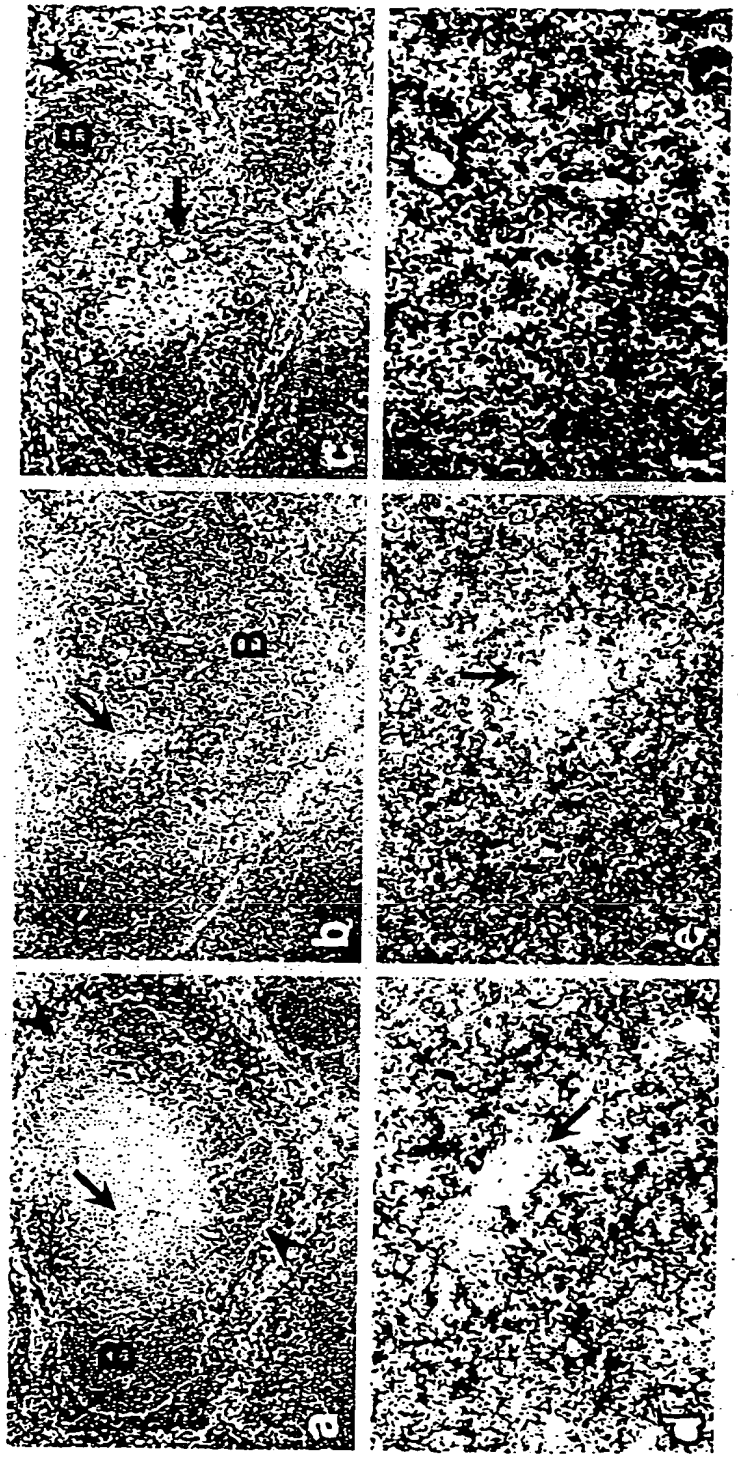


FIGURE 22

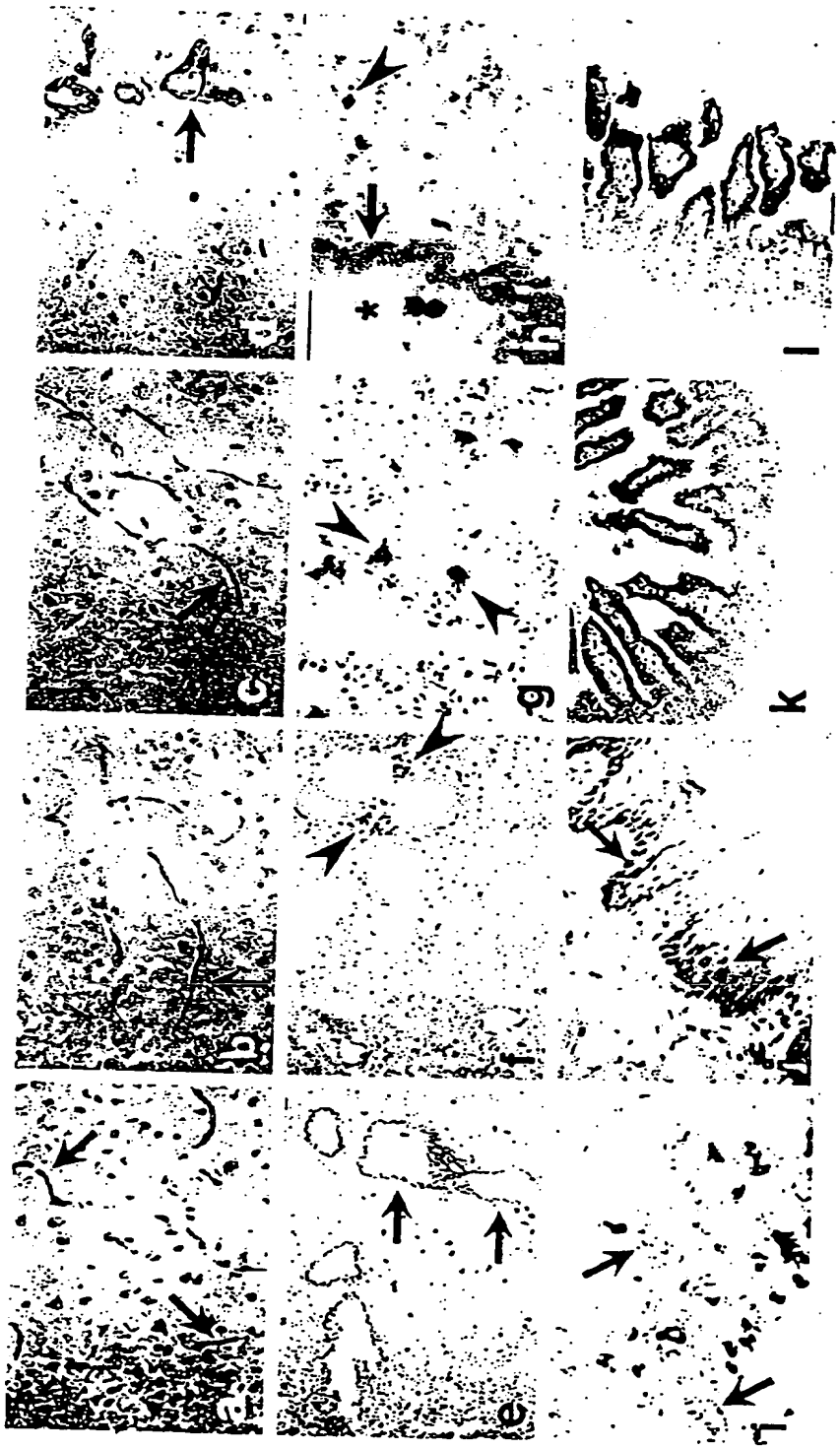
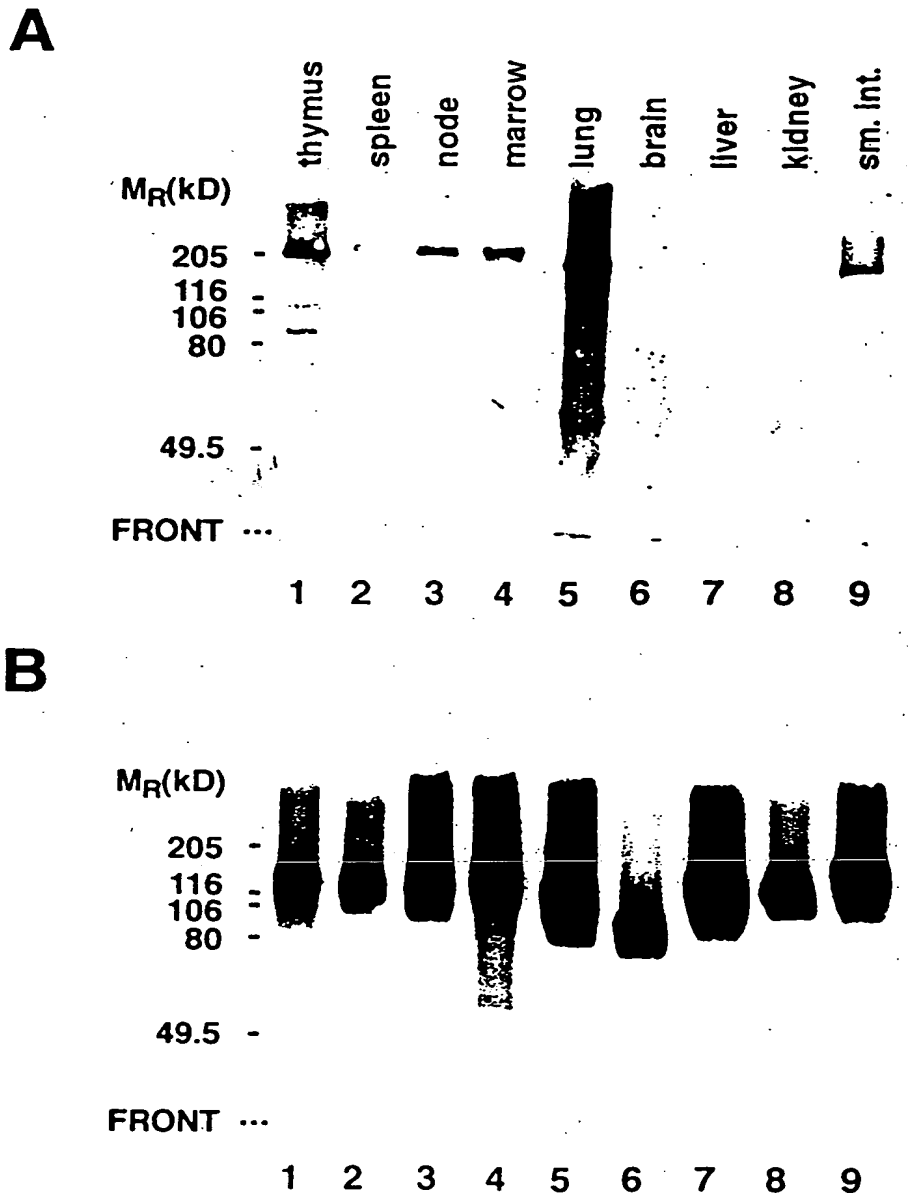


FIGURE 23


A
thymus
spleen
node
marrow
lung
brain
liver
kidney
sm. int.
M_R(kD)
205
116
106
80
49.5
FRONT
1 2 3 4 5 6 7 8 9
B
M_R(kD)
205
116
106
80
49.5
FRONT
1 2 3 4 5 6 7 8 9